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Conservation genetics of American crocodile, *Crocodylus acutus*, populations in Pacific Costa Rica

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Abstract

Maintaining genetic diversity is crucial for the survival and management of threatened and endangered species. In this study, we analyzed genetic diversity and population genetic structure at neutral loci in American crocodiles, *Crocodylus acutus*, from several areas (Parque Nacional Marino Las Baulas, Parque Nacional Santa Rosa, Parque Nacional Palo Verde, Rio Tarcoles, and Osa Conservation Area) in Pacific Costa Rica. We genotyped 184 individuals at nine microsatellite loci to describe the genetic diversity and conservation genetics between and among populations. No population was at Hardy-Weinberg Equilibrium (HWE) over all loci tested and a small to moderate amount of inbreeding was present. Populations along the Pacific coast had an average heterozygosity of 0.572 across all loci. All populations were significantly differentiated from each other with both F_{ST} and R_{ST} measures of population differentiation with a greater degree of molecular variance (81%) found within populations. Our results suggest *C. acutus* populations in Pacific Costa Rica were not panmictic with moderate levels of genetic diversity. An effective management plan that maintains the connectivity between clusters is critical to the success of *C. acutus* in Pacific Costa Rica.

Keywords

American crocodile, heterozygosity, microsatellites, population genetics, genetic structure

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Introduction

Threatened and endangered species face many challenges including habitat fragmentation and destruction, human population growth, and loss of genetic variability. Maintenance of genetic diversity is of increasing importance in the preservation of threatened and endangered species (Lacy 1997; Haig 1998; Reed and Frankham 2003; Reed et al. 2007). Lack of genetic diversity can lead to inbreeding depression (Frankham 1995), decreased immunity (O'Brien et al. 1985), decreased reproductive performance (O'Brien et al. 1985; Parker et al. 1991) and eventual extinction (Frankham 2005). Effective management strategies for threatened and endangered species require integration of all aspects of the species' biology, including both demography and genetics (Lande 1988)

The American crocodile (*Crocodylus acutus*) is mainly a coastal species ranging from the extreme southern tip of Florida, through the Caribbean, and Central and northern South America (Mazzotti 1999; Thorbjarnarson 2010). Populations rangewide are threatened by habitat destruction and fragmentation, poaching, and past overexploitation (Ross 1998; Thorbjarnarson et al. 2006; Mazzotti et al. 2007). Current threats to *C. acutus* in Costa Rica include habitat loss (particularly nesting habitat), deliberate killing (Machkour-M'Rabet et al. 2009), and pollution (Rainwater et al. 2007; Rainwater et al. 2011). *Crocodylus acutus* is a wide ranging and ecologically plastic species (Thorbjarnarson 2010) with substantial genetic differentiation among populations (Menzies and Kushlan 1991; Rodriguez 2007; Porras et al. 2008; Thorbjarnarson 2010). Determining the status and ecology of *C. acutus* in Costa Rica is a priority project of the IUCN Crocodile Specialist Group Action Plan (Ross 1998). Genetic evaluations of *C. acutus* range-wide were further named as a moderate priority project in the 2010 Action Plan (Thorbjarnarson 2010). Therefore, it is important to describe genetic diversity and differentiation of this species throughout its range, including Costa Rica.

In the present study, we investigated the genetic structure of *C. acutus* populations in several areas of Pacific Costa Rica (Fig. 1) and the degree of effective migration occurring between populations. A series of estuaries provide pockets of suitable crocodile habitat along the Pacific coast which made it an optimal area for studying gene flow between potential metapopulations. Crocodiles are known to migrate long distances (Kay 2004; Campos et al. 2006; Read et al. 2007; Campbell et al. 2013). There have been accounts of *C. acutus* migrating over 35 km for nesting (Cherkiss et al. 2006) and movements over 388 km (Cherkiss et al. 2014) in southern Florida. The ability of crocodilians to migrate and disperse long distances increases the amount of potential gene flow between neighboring or distant populations. It is possible that crocodiles are dispersing between habitat patches in Pacific Costa Rica; thus, facilitating gene flow along the coast. We used microsatellites to test the hypothesis that *C. acutus* populations do not exist as a continuous, panmictic population in Pacific Costa Rica.

Methods

Study area

We sampled 5 localities in Pacific Costa Rica for *C. acutus* (Fig. 1). Site LB (Parque Nacional Marino Las Baulas) was in the Tempisque Conservation Area (ACT); site PV (Parque Nacional Palo Verde) was in the Arenal-Tempisque Conservation Area (ACA-T); site SR (Parque Nacional Santa Rosa) was in the Guanacaste Conservation Area (ACG); site RT (Rio Tarcoles) was in Central Pacific Conservation Area (ACOPAC); sites RS (Rio Sierpe), T (Terraba Delta), PL (Pejeperro Lagoon), PTL (Pejeperrito Lagoon), RE (Rio Esquinas), RC (Rio Coto) and PB (Parrot Bay Lodge, Puerto Jimenez) were in the Osa Conservation Area (ACOSA). Crocodiles were sampled from seven areas in ACOSA; however, they have been combined as one population due to low sample numbers. Localities ranged from large river systems (PV, RT, and ACOSA), to estuaries (LB and SR) and coastal lagoons (SR and ACOSA). (See Mauger et al. 2012 for additional study location information.)

Sample collection

We collected blood and tissue samples at the beginning of the rainy season in SR (2007) and PV (2005, 2008 and 2009), throughout the year in LB (2007 – 2009), during the rainy season in RT (2005 – 2006) and during the end of the dry season in ACOSA (2006, 2008 and 2009). We captured crocodiles mainly during spotlight surveys using the break-away snare method (Hutton et al. 1987; Hutton and Woodhouse 1989), snake tongs or by hand (see Mauger et al. 2012 for additional information on sample collection). Blood and/or tissue was collected from 184 individuals (see Table 1 for size class distribution of samples). In sites where a large number of hatchlings were captured, a random number selector was used to randomly select four to six hatchlings for genetic analysis. Tissue was collected from the caudal scutes during marking and blood was collected from the caudal vein or the dorsal sinus. Tissue samples were preserved in 95–100% ethanol. Blood was preserved on Whatman FTA Cards for DNA Preservation® (GE Life Sciences).

DNA isolation and microsatellite amplification

We isolated DNA from tissue samples using the DNeasy Blood and Tissue Kit™ (Qiagen) and purified from blood cards with two five-minute washes with FTA Purification Reagent (Whatman) and two five-minute washes with Tris-EDTA (TE; 10 mM Tris-Cl, pH 7.5, 1 mM EDTA) buffer. Each wash consisted of 50 µl of solution.

We amplified nine microsatellite DNA loci using previously characterized primers (Dever and Densmore 2001; Fitzsimmons et al. 2001) C391, Cj16, Cj18, Cj20,

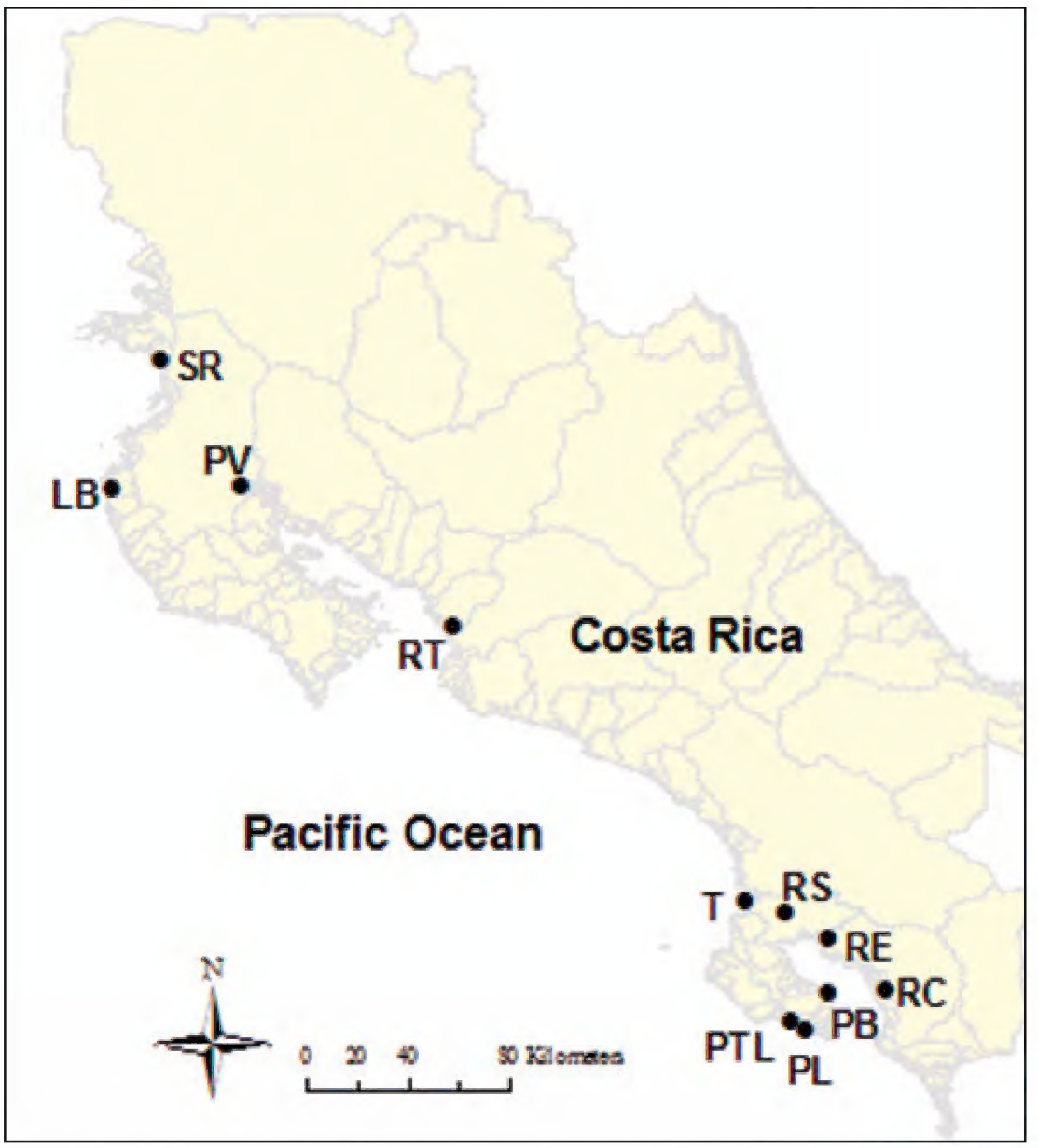

Figure 1. Map of collection sites in Costa Rica of the American crocodile, *Crocodylus acutus*. Parque Nacional Marino Las Baulas (LB), Parque Nacional Palo Verde (PV), Parque Nacional Santa Rosa (SR), Rio Tarcoles (RT), Rio Sierpe (RS), Terraba Delta (T), Pejeperitto Lagoon (PTL), Pejeperro Lagoon (PL), Rio Esquinas (RE), Rio Coto (RC) and Parrot Bay Lodge (PB). Sites RS, T, PTL, PL, RE, RC, and PB were grouped together as Osa Conservation Area (ACOSA). Localities ranged from large river systems (PV, RT, and ACOSA) to estuaries (LB, SR) and coastal lagoons (SR and ACOSA).

Table 1. Size class distribution of genotyped crocodiles for each site.

Site	Hatchling	Juvenile	Subadult	Adult	Total
LB	12	17	13	4	46
SR	4	8	5	0	17
ACOSA	0	44	3	1	48
PV	9	32	2	9	53
RT	0	13	0	3	19
Total	25	114	23	17	184

Cj109, Cj131, CU5-123, CUD68, and CUJ131 via polymerase chain reaction (PCR). The forward primer of each pair was labeled with a fluorescent dye (6-FAM, HEX or NED; Applied Biosystems) to allow for the detection and sizing of DNA fragments.

The DNA was amplified in 25 µl reactions containing 1.25 units of EconoTaq DNA Polymerase (Lucigen), 2.5 µl 10X buffer (100 mM Tris-HCl (pH 9.0), 500 mM KCl, 1% Triton X-100, 15 mM $MgCl_2$), 1.0 µl 25 mM $MgCl_2$ (Cj16 and Cj20) or 0.5 µl 25 mM $MgCl_2$ (all other primers), 1.0 µl of 10 mM dNTP's (Qiagen), 1.0 µl each of the forward and reverse primer, approximately 100 ng template DNA and purified water to the final volume. Microsatellites were amplified according to the following parameters: initial denaturation at 94°C for 2 minutes, 33 cycles of 94°C for 1 minute, 58°C (C391, Cj18, Cj131, CU5-123, CUD68, CUF131), 59°C (Cj16, Cj20) or 62°C (Cj109) for 1 minute, and 72°C for l minute, and a final extension at 72°C for 10 minutes. Amplified loci were separated on an Applied Biosystems (ABI) 3730xl Genetic Analyzer and sized with LIZ-500 size standard by Genewiz, Inc (www.genewiz.com). Genotypes were assigned using PeakScanner 1.0 (Applied Biosystems).

Genetic diversity

Data files were converted to formats supported by various genetic programs in CREATE 1.0 (Coombs et al. 2007). Probability of Identity (PI) was estimated in GENALEX 6 (Peakall and Smouse 2006) to determine the minimum number of microsatellites needed to identify individuals. Allelic richness (A_R) and the number of private alleles (A_{Priv}) were estimated in HP-RARE (Kalinowski 2005). We estimated numbers of alleles, allele frequencies and gene diversities in FSTAT 2.9.3.2 (Goudet 1995).

Observed versus expected number of heterozygotes were estimated in Genepop on the Web (Raymond and Rousset 1995; Rousset 2008). Departure from Hardy-Weinberg Equilibrium (HWE) and linkage disequilibrium (LD) were estimated in Genepop on the Web (Raymond and Rousset 1995; Rousset 2008). Departure from HWE was tested using an exact test (Guo and Thompson 1992) and a chi-square goodness of fit test with a dememorization number of 10,000, and 1,000 batches of 10,000 iterations each. Linkage disequilibrium was tested to determine if small effective population sizes within the different localities caused nonrandom association of alleles at different loci. Linkage disequilibrium was tested for all pairs of loci used by the log likelihood ratio statistic under the same parameters as HWE. All p-values were adjusted to allow for multiple comparisons. Weir and Cockerham's (1984) inbreeding coefficient, F_{IS}, was estimated for each population in FSTAT 2.9.3.2 (Goudet 1995) with and without randomly selected hatchlings. Homozygote excess at each locus in each locality was estimated by MICROCHECKER (Van Oosterhout et al. 2004). MICROCHECKER was also used to identify if null alleles were present at each locus in each locality. Null alleles were suggested for loci with a general excess of homozygotes for most allele size classes.

We identified whether rare alleles had been lost due to previous genetic bottlenecks under the infinite alleles (IAM) and stepwise mutation (SMM) models in BOTTLENECK version 1.2.02 (Piry et al 1999). Both the standardized differences and Wilcoxon tests were run.

Population genetic structure

We used an analysis of molecular variance (AMOVA) to estimate the percentage of variance within and among populations with GENALEX 6 (Peakall and Smouse 2006). Population differentiation was estimated for all population pairs using several methods. We estimated F_{ST} and R_{ST} for each population pair in FSTAT 2.9.3.2 (Goudet 1995) and Arlequin ver. 3.11 (Excoffier et al. 2005) respectively. All p-values were adjusted to allow for multiple comparisons.

We used Mantel's test to determine the relationship between geographic and genetic distance. Isolation by Distance Web Service (IBDWS; Jensen et al. 2005) was used to test for the presence of isolation by distance (IBD) between population pairs. Sites RT and T were excluded from the analysis because GPS coordinates of crocodile captures were not available. Each Mantel test was performed with 30,000 randomizations. Distances between populations were estimated using an oceanic/coastline route. Rousset's genetic distance (F/(1-F)) was calculated using genetic differentiation (F_{ST}).

Results

Genetic diversity

The nine microsatellites chosen for this study had an average probability of identity (PI) of 4.96^{-6} across all five Costa Rican populations (SR=5.4^{-6}; LB=7.9^{-6}; ACOSA=8.7^{-8}; PV=1.5^{-7}; RT=1.1^{-5}). This indicated that there was a low probability that two individuals chosen at random would have the same genotype. These microsatellites were sufficient for this study.

We identified 88 alleles in five *C. acutus* populations sampled in Pacific Costa Rica across all nine microsatellite loci. Average A_R and A_{Priv} over all loci were estimated using a corrected sample size of 34 alleles. The A_R ranged between 4.22 and 5.64 and A_{Priv} ranged between 0.27 and 1.36 in the sampled locations (Table 2). Allele frequencies for each microsatellite locus ranged from 0 (in localities where the allele was not genotyped) to 0.882 (Appendix 1: Allele Frequencies). Allele frequencies were also calculated with the hatchlings removed. There were no substantial differences in allele frequencies when hatchlings were removed. We tested for genetic bottlenecks under the IAM and SMM models for all samples combined and each site separately. Bottlenecks were not detected under IAM ($p>0.05$), but were detected in all populations under SMM ($p<0.009$) with the standardized differences test.

No population was in Hardy-Weinberg Equilibrium (HWE) over all nine microsatellite loci tested (Table 3). Site LB was not in HWE at loci Cj16 ($p<0.001$), Cj109 ($p=0.03$) and Cj131 ($p=0.01$); site SR was not in HWE equilibrium at locus C391 ($p=0.005$); site ACOSA was not in HWE at loci C391 ($p=0.03$), Cj18 ($p=0.02$), Cj20 ($p=0.04$), Cj109 ($p=0.002$), CU5-123 ($p<0.001$) and CUD68 ($p<0.001$); site PV was not in HWE at loci Cj18 ($p=0.001$), Cj109 ($p=0.03$), Cj131 ($p=0.01$), CU5-123

Table 2. Genetic variability estimates for *Crocodylus acutus* populations in Pacific Costa Rica.

Sample Site	Code	N	A_R	A_{Priv}
Area of Conservation Tempisque	ACT			
Las Baulas National Park	LB	46	4.31	0.37
Palo Verde National Park	PV	54	5.19	0.58
Area of Conservation Guanacaste	ACG			
Santa Rosa National Park	SR	17	4.22	0.3
Central Pacific Conservation Area	ACOPAC			
Rio Tarcoles	RT	17	4.22	0.27
Osa Conservation Area	ACOSA	49	5.64	1.36

N = sample size.

A_R = allelic richness.

A_{Priv} = number of private alleles.

Table 3. Expected (H_E) and observed heterozygosities (H_O) for microsatellite loci in *Crocodylus acutus* populations.

	LB (N = 46)		**SR (N = 17)**		**PV (N = 54)**		**RT (N = 17)**		**ACOSA (N = 49)**	
Locus	H_E	H_O	H_E	H_O	H_E	H_O	H_E	H_O	H_E	H_O
C391	0.46	0.52	0.65#	0.35*+	0.59	0.57	0.36	0.35	0.57#	0.53*
Cj16	0.48	0.28*+#	0.51	0.53	0.62	0.59	0.39	0.47	0.64	0.65
Cj20	0.41	0.61	0.56	0.35	0.67	0.59	0.75#	0.41*	0.66#	0.63+
Cj131	0.49	0.48	0.22	0.24	0.37#	0.43	0.39	0.35	0.45	0.47*
Cj18	0.45	0.41	0.47	0.53	0.76#	0.70*	0.73#	0.41*+	0.89#	0.80*+
Cj109	0.64	0.59*#	0.78	0.65	0.78#	0.67*	0.62	0.76+	0.61#	0.39*
CU5-123	0.40	0.37*	0.06	0.06	0.62#	0.33*+	0.49	0.53+	0.45#	0.29*
CUD68	0.70	0.54*+	0.68	0.65	0.73#	0.46+	0.65#	0.35*+	0.76#	0.39*+
CUJ131	0.68	0.67#	0.63	0.41*	0.57#	0.61	0.56	0.76	0.54	0.51

*heterozygote deficiency at these loci (Wilcoxon test; p=0.05).

+homozygote excess at these loci suggested that null alleles may be present (p=0.05).

#loci removed from Hardy-Weinberg Equilibrium.

(p<0.001), CUD68 (p<0.001) and CUJ131 (p=0.02); and site RT was not in HWE at loci Cj18 (p=0.03), Cj20 (p=0.02) and CUD68 (p=0.0026). The Wilcoxon test identified heterozygote deficiencies in all localities for at least one microsatellite loci (p=0.002-0.02; Table 3). Null alleles were suggested for at least one locus in each locality by general excess of homozygote for most allele size classes (Table 3). Average F_{IS} values ranged from 0.096 to 0.179 for each site. Inbreeding levels were 0.179 and 0.103 in ACOSA and RT, respectively. Inbreeding coefficients were calculated with hatchlings included and removed in the remaining sites. Site PV had a lower F_{IS} when hatchlings were included (0.127 and 0.142 with and without hatchlings, respectively). Site SR had higher F_{IS} when hatchlings were included (0.179 and 0.166 with and without hatchlings, respectively). There was no difference in F_{IS} (0.096) in site LB.

Linkage disequilibrium (LD) tests were performed to investigate the distribution of the nine microsatellite loci for *C. acutus* populations on the Pacific coast of Costa Rica. Pairwise comparisons were performed for each population. LD did not play a strong role in the nine microsatellites tested (p=0.001-0.99). All p values were adjusted for multiple tests.

Population genetic structure

An analysis of molecular variance estimated that 19% of the variation occurred between populations, while 81% of molecular variance occurred within individual populations. This suggested that individual populations were genetically diverse. Population differentiation was measured between all population pairs using F_{ST} and R_{ST}. All population pairs were significantly differentiated (p=0.05) using both measures of population differentiation (Table 4). We observed the least amount of differentiation between ACOSA with LB and SR and the highest level of differentiation between LB with SR and RT.

Isolation by distance (IBD) was estimated between each sampled crocodile in all localities in Pacific Costa Rica. No IBD was observed (p=0.92; Fig. 2). Isolation between populations did not restrict gene flow.

Discussion

The nine microsatellites chosen in this study provided data on the genetic structure of *C. acutus* populations along the Pacific coast of Costa Rica. Average heterozygosity of crocodiles along the Pacific coast of Costa Rica was slightly higher than or comparable to that in other crocodilian populations (Glenn et al. 1998; Davis et al. 2001; Dever et al. 2002; Ryberg et al. 2002; Verdade et al 2002; de Thoisy et al. 2006; Rodriguez et al. 2008). However, in this study, several individual loci did have lower heterozygosity values, possibly due to inbreeding observed in all populations. Crocodiles within site SR had higher F_{IS} values than other sites. This may be because we observed and captured few individuals that had exceeded minimum breeding size. This site also represented the smallest crocodile habitat with lower encounter rates (Mauger et al. 2012). Fewer breeding crocodiles and lower encounter rates could explain the higher inbreeding levels since presumably the gene pool is limited to fewer individuals. In all surveyed localities, size class distributions estimated during the study showed a higher percentage of juveniles (0.5 – 1.25 m) than adults (>2.25 m) (Mauger et al. 2012). The higher percentage of juveniles in these localities could indicate population recovering from past bottlenecks (Ouboter and Nanhoe 1989) and explain the higher inbreeding levels observed in this study.

We identified previous genetic bottlenecks in all sampled localities. Past bottlenecks were confirmed under the SMM model by BOTTLENECK (Piry et al. 1999).

Table 4. Population differentiation between all *Crocodylus acutus* population pairs using R_{ST} (above 0 line) and F_{ST} (below 0 line).

Site	LB	SR	ACOSA	PV	RT
LB	–	0.66⁺	0.04⁺	0.23⁺	0.65⁺
SR	0.19⁺	–	0.04*	0.18⁺	0.11*
ACOSA	0.15⁺	0.1⁺	–	0.10⁺	0.06*
PV	0.1⁺	0.08⁺	0.07⁺	–	0.24⁺
RT	0.24⁺	0.14⁺	0.10⁺	0.10⁺	–

*significant at p=0.05

⁺significant at p=0.001

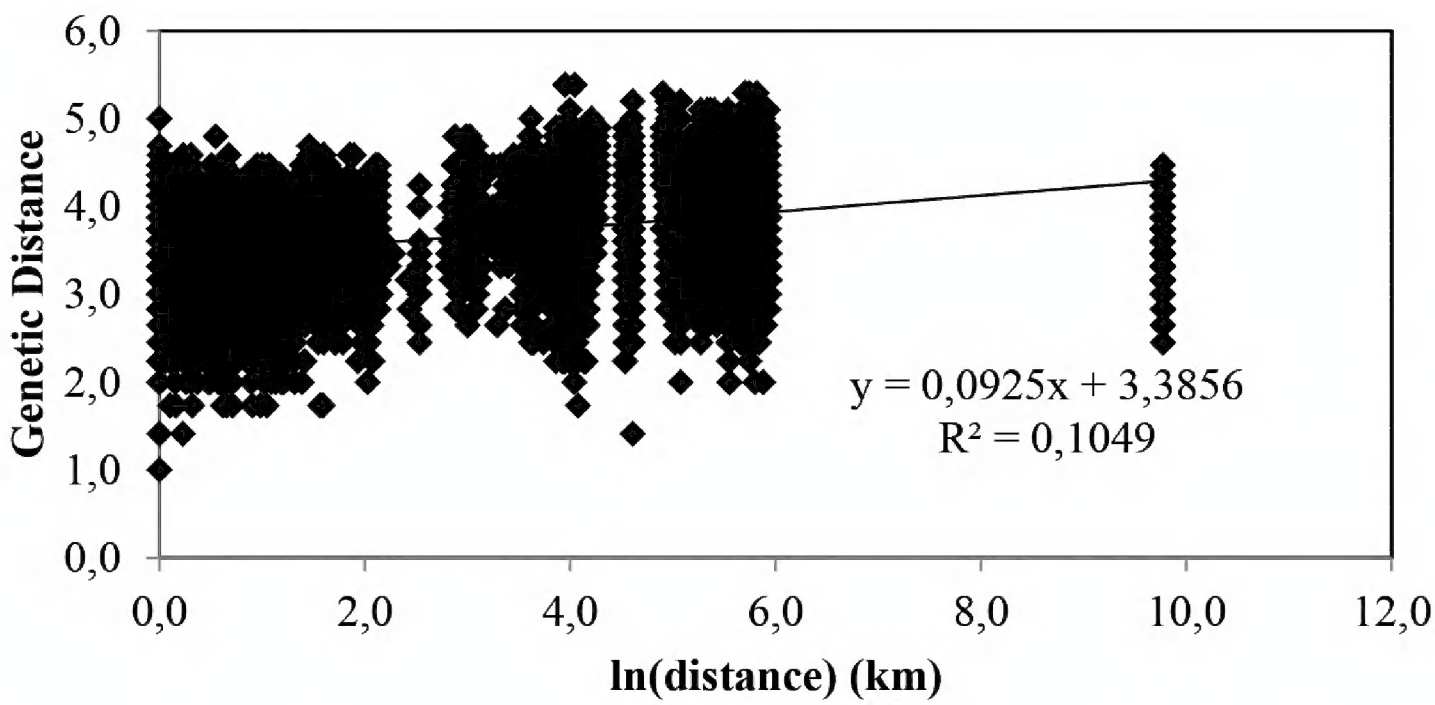

Figure 2. Mantel test for isolation by distance between individual crocodiles in all localities. No isolation by distance was observed (p=0.92). Rousset's distance (F/(1-F)) was used for genetic distance. Geographic distance was the log of a straight line distance (km) between populations.

Based on these analyses, we concluded that all populations underwent a previous reduction in population size. Population bottlenecks lead to the rapid loss of rare alleles and results in the loss of the total number of alleles at a faster rate than a loss in overall heterozygosity (Ortego et al. 2010). The low number of private alleles observed in this study (Table 2) could be an artifact of past genetic bottlenecks. Null alleles were also detected for at least one locus in each locality (Table 3). The absence of these alleles in the analysis could help explain the loss of genetic variation observed in these localities. *Crocodylus acutus* populations declined range-wide through the mid-20th century as a result of hunting and illegal poaching (Ross 1998; Thorbjarnarson et al. 2006; Thorbjarnarson 2010). These human activities have not been documented in Costa Rica; however, it is possible that populations here experienced similar pressures and population declines. We observed some poaching and killing of large individuals in LB during the study and have received reports of similar activities at other sites in Pacific

Costa Rica (personal observation and communication with local people). Anecdotal data suggests that crocodile numbers are also increasing in LB (F. Paladino, personal communication) and PV (Bolaños-Montero 2012). Recent introduction of tilapia into the Tempisque River Basin (site PV) has provided a continuous food source for crocodiles (Sandlund et al. 2010), potentially contributing to the recent population growth. As a result, crocodile numbers in this region have increased precipitously in recent years, causing adverse interactions between human and crocodile populations (Bolaños-Montero 2012). Additional factors are most likely at play, which are contributing to population growth.

No population was in Hardy-Weinberg Equilibrium for all microsatellite loci. This could be due to the heterozygote deficiency observed at loci that were not in HWE, to inbreeding documented in several populations, or to effective migration. Our results suggest that crocodile populations in Pacific Costa Rica were differentiated from each other, as supported by studies on *C. acutus* and other crocodilian species (Farias et al. 2004; Porras et al. 2008; Machkour-M'Rabet et al. 2009; Thorbjarnarson 2010). All population pairs exhibited significant genetic differentiation, with all but two population pairs (LB and ACOSA, and SR and ACOSA) showing moderate differentiation from each other (R_{ST}<0.05; Table 4). These values supported the hypothesis that migration occurred between populations; however, some population pairs were more differentiated from each other and thus had lower historical migration rates between patchily distributed habitats in Costa Rica. The level of subdivision observed suggested that crocodiles in Pacific Costa Rica were not panmictic; however, genetic connections did exist. Additionally, the majority of molecular variance was observed within populations. This could explain why moderate differentiation was observed between most population pairs. However, the highly mobile nature of crocodiles (Kay 2004; Campos et al. 2006; Read et al. 2007; Campbell et al. 2013) could be facilitating gene flow between populations along the Pacific coast. A recent study on the spatial ecology of *C. acutus* in Panama, suggests that males have a larger home ranges, but females have larger average movement distances (Balaguera-Reina et al. 2016). Balaguera-Reina et al. (2016) also noted dispersal differences between age classes and dry and wet seasons. Dispersal differences between age classes, i.e. subadults dispersing to find mating territories, could also explain the departure from Hardy-Weinberg Equilibrium and moderate differentiation levels. GPS-based tracking studies of Costa Rican *C. acutus* would contribute important information on contemporary crocodile dispersal abilities and maximum home ranges in patchily distributed habitats.

Conclusions

The data presented here supported moderate differentiation and an absence of isolation by distance in Pacific Costa Rica. Our results suggested the loss of genetic variation through a lack of connectivity between some localities and previous population bottlenecks. The moderate heterozygosity values and genetic differentiation described

here emphasized the need to protect all potential crocodile habitat, to write management plans across conservation areas and national parks in Costa Rica, and the need for conservation and management units to extend over the entire span of a species' range.

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Appendix I

Allele Frequencies. Allele frequencies for each locus in each *Crocodylus acutus* population studied in Pacific Costa Rica. The number in parenthesis indicates the sample size in that location.

Locus	Allele	LB (N=46)	SR (N=17)	PV (N=54)	RT (N=17)	ACOSA (N=49)
C391	139	0	0	0.009	0	0
	141	0.022	0	0.093	0.088	0.02
	143	0.011	0	0	0	0
	147	0.022	0.029	0	0	0
	149	0.065	0.059	0.028	0	0.041
	151	0.033	0.206	0.037	0	0.02
	153	0.728	0.559	0.611	0.794	0.602
	155	0.022	0.029	0	0	0.031
	157	0	0.029	0.167	0.088	0.265
	159	0	0	0.009	0	0
	161	0	0.088	0.046	0.029	0.01
	163	0.098	0	0	0	0.01
Cj16	151	0	0	0	0	0.031
	153	0	0.676	0.287	0.765	0.551
	155	0	0	0.009	0	0.041
	157	0	0	0	0	0.01
	173	0	0	0.009	0.029	0
	175	0.13	0.029	0.093	0.176	0.02
	183	0	0	0.019	0	0.071

Locus	Allele	LB (N=46)	SR (N=17)	PV (N=54)	RT (N=17)	ACOSA (N=49)
Cj16	185	0.696	0.147	0.537	0	0.224
	187	0.163	0.147	0.046	0.029	0.051
	189	0.011	0	0	0	0
Cj18	195	0	0	0	0	0.02
	197	0.011	0	0	0	0
	199	0.054	0	0.157	0.088	0.071
	201	0.739	0.029	0.231	0.147	0.133
	203	0	0	0	0.176	0.041
	205	0	0	0	0	0.01
	215	0.043	0	0	0	0.163
	217	0.054	0.059	0.046	0.029	0.112
	219	0.011	0.206	0.111	0.088	0.01
	221	0.054	0	0.019	0	0.041
	223	0.033	0.706	0.389	0.471	0.204
	225	0	0	0.037	0	0.031
	227	0	0	0	0	0.092
	229	0	0	0.009	0	0.071
Cj20	168	0.011	0	0	0	0.031
	170	0.022	0.059	0.019	0.029	0.02
	172	0.054	0.265	0.296	0.206	0.51
	174	0.75	0.618	0.491	0.206	0.143
	176	0	0.029	0.037	0.118	0
	178	0.163	0.029	0.074	0.412	0.245
	186	0	0	0.046	0	0
	196	0	0	0	0	0.051
	200	0	0	0	0.029	0
	206	0	0	0.019	0	0
	212	0	0	0.019	0	0
Cj109	364	0	0	0.065	0.029	0
	366	0.054	0.176	0.343	0.412	0.214
	368	0.511	0.324	0.194	0.059	0.551
	370	0.141	0.235	0.231	0.471	0.204
	372	0	0.029	0.037	0.029	0.01
	374	0.293	0.235	0.12	0	0.01
	376	0	0	0.009	0	0
	378	0	0	0	0	0.01
Cj131	209	0	0.029	0	0	0
	211	0.087	0	0.019	0.059	0.061
	213	0.141	0.059	0.194	0.176	0.143
	215	0.696	0.882	0.769	0.765	0.724
	217	0.065	0.029	0	0	0.051
	219	0.011	0	0.019	0	0
	231	0	0	0	0	0.02

Locus	Allele	LB (N=46)	SR (N=17)	PV (N=54)	RT (N=17)	ACOSA (N=49)
CU5-123	218	0	0	0.01	0	0.013
	220	0	0	0.029	0	0
	222	0.011	0	0	0	0
	224	0.189	0	0.279	0	0.079
	226	0.744	0.971	0.519	0.618	0.605
	228	0.033	0.029	0.106	0	0.026
	230	0.022	0	0.01	0	0
	232	0	0	0	0	0.066
	234	0	0	0.048	0.382	0.211
	220	0	0	0.029	0	0
	222	0.011	0	0	0	0
	224	0.189	0	0.279	0	0.079
	226	0.744	0.971	0.519	0.618	0.605
	228	0.033	0.029	0.106	0	0.026
	230	0.022	0	0.01	0	0
	232	0	0	0	0	0.066
	234	0	0	0.048	0.382	0.211
CUD68	105	0	0	0	0	0.031
	121	0	0	0	0	0.02
	123	0.054	0	0.009	0	0.051
	125	0.261	0.294	0.389	0.029	0.173
	127	0.348	0	0.139	0.147	0.367
	129	0.337	0.412	0.231	0.353	0.265
	131	0	0	0.231	0.471	0.092
	133	0	0.294	0	0	0
CUJ131	141	0.011	0	0	0	0
	155	0	0	0.019	0.059	0
	171	0.272	0.088	0.009	0.265	0
	175	0	0	0.009	0	0.01
	179	0.011	0.059	0.009	0	0.02
	181	0.337	0.5	0.389	0.059	0.582
	183	0	0	0.028	0	0.031
	185	0.37	0.353	0.528	0.618	0.357
	187	0	0	0.009	0	0